ANNUAL PROGRESS REPORT

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Contract:	Nonr - 994(00)
Rate:	$3,080.00
Contractor:	The University of Texas
Principal Investigator:	Gerald R. Seaman
Title of Project:	Research on Adaptive Metabolism of Protozoa.

SUMMARY OF RESULTS:

A subculture of T. pyriformis S has been established in media lacking in added riboflavin and thiamine. Microbiological assays indicate synthesis of the omitted vitamins by the ciliates. Ability to synthesize lysine, arginine, and methionine, as well as riboflavin, has been attained by a second subculture. Synthesis of the amino acids by this culture has been demonstrated by quantitative paper chromatography.

Homogenates of cells grown in the absence of added thiamine synthesize the vitamin in vitro from 2-methyl-5-hydroxymethyl-6-aminopyrimidine and 4-methyl-5-(β-hydroxymethyl)-thiazole. Although methylene blue, ATP, and high concentrates of Mg^{++} and Ca^{++} were included in these incubation mixtures, the absolute requirements for the reaction have not been ascertained.

In addition to being required for the oxidation of α-keto acids, thioctic acid has been found to be required for the oxidation of acetate according to the equation:

ATP + CoA + acetate ⟶ acetyl-CoA + pyrophosphate + AMP

These experiments have been carried out with enzymes prepared from liver of dog, beef, duck, and pigeon.

The acetate-activating system from vertebrate tissues has been separated by ammonium sulfate precipitation. One fraction contains the acetate-activating enzymes, but does not respond to the procedure for removing thioctic acid from enzymes. A second fraction, which in itself

exhibits only very slight activating activity, when added to the first fraction, enables removal of thioctic acid. It therefore appears that the second fraction functions by splitting bound thioctic acid from the enzyme protein; liberated thioctic is then adsorbed and removed in the procedure. Evidence has also been obtained for the presence of enzymes necessary for "activation" of thioctic acid in the acetate reaction.

PLANS FOR THE FUTURE:

The requirements for the *in vitro* synthesis of thiamine by *Tetrahymena* will be ascertained. In addition, enzymatic synthesis of the thiazole and pyrimidine moieties will be attempted. *In vitro* syntheses will be examined for other nutrients with enzymes prepared from appropriately "deficient" cells.

Further investigations will be undertaken to determine the generality of the thioctic acid requirement in acyl transferring reactions and additional investigations of the mechanism of the "thioctic splitting" and "thioctic activation" enzymes should provide valuable insight as to the active coenzyme form of the vitamin.

REPORTS:

Participation of Thioctic Acid in the Acetate-Activating Reaction. Presented at the B Vitamin Symposium, University of Texas, Austin, Texas, December 2-5, 1953.